IX meeting
Stem Cell Research Italy

PAOLO BIANCO AWARD

SCIENTIFIC COMMITTEE
Umberto Galderisi
Yvan Torrente

JUNE 21 - 22 - 23, 2018

Milano - ITALY

AULA MAGNA DELL'UNIVERSITÀ DEGLI STUDI DI MILANO
VIA FESTA DEL PERDONO, 7

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21 THURSDAY JUNE

14.15 **Attendee registration**

14.45 **WELCOME CEREMONY:**
Umberto Galderisi and Yvan Torrente

NEW HORIZONS FOR STEM CELL RESEARCH: iPS, EXTRACELLULAR VESICLES AND BEYOND
Chair: ***Augusto Pessina***

KEY LECTURE

15.00 ***Maurilio Sampaolesi***
miRNAs, exosomes and iPS cell technologies

15.45 ***Anna Lange-Consiglio***
Anti-inflammatory and immunomodulatory miRNA cargo of microvesicles obtained from equine amniotic progenitor cells in different time-span of in vitro culture

16.15 **TWO ORAL COMMUNICATIONS BY YOUNG INVESTIGATORS**
Chairs: ***Assunta Pandolfi and Letizia Penolazzi***

Donato Cappetta
Lung mesenchymal stem cells ameliorate elastase-induced damage in an animal model of emphysema

Sara Patruno
Human periodontal ligament stem cell differentiation into airway cells: New perspectives for studies on lung diseases

16.45 *Coffee break*

PAOLO BIANCO AWARD
Chairs: ***Assunta Pandolfi and Letizia Penolazzi***

17.15 **THREE SELECTED COMMUNICATIONS BY YOUNG INVESTIGATORS**

Andrea Brambilla
Myoexosomes cargo triggers muscle regeneration and provides molecular cues for alternative exon skipping theraphy in muscular dystrophy

Maria Magdalena Barreca
Oxidative stress induces necroptosis in mouse mesoangioblast stem cells

Nefele Giarratana
MICAL2 affects myogenic differentiation

18.00 **SNAPSHOTS BY COMPANIES**

Welcome cocktail

22 FRIDAY JUNE

08.30 **Attendee registration and poster set-up**

STEM CELLS IN NEUROMUSCULAR AND CARDIAC DISEASES
Chairs: ***Marco Marchisio and Domitilla Mandatori***

09.00 ***Davide Gabellini***
Molecular pathogenesis of FSHD muscular dystrophy

09.30 ***Sveva Bollini***
Paracrine boosting of endogenous mechanisms of cardiac regeneration

10.00 ***Massimiliano Gnecchi***
Induced pluripotent stem cells technology: toward precision medicine in cardiology

10.30 *Coffee break*

POSTER SESSION
Chairs: *Yvan Torrente and Roberta Piva*

11.00 *Guided Poster Tour (5 minutes presentation)*

12.00 *SCR Assembly*

13.00 *Light lunch*

FROM BASIC TO APPLIED RESEARCH IN STEM CELL FIELD: AN UPDATE
Chairs: *Roberta Piva and Tiziana Squillaro*

14.00 *Lorenza Lazzari*
Can we distinguish fetal, adult and iPS-derived MSCs?

14.30 *Margherita Maioli*
Unfolding the stem cell world: a path among the use of molecules and physical energies

15.00 *Gianvito Martino*
Therapeutic plasticity of neural stem cells

15.30 **THREE ORAL COMMUNICATIONS BY YOUNG INVESTIGATORS**
Chairs: *Mario Romano and Elisabetta Lambertini*

Ilenia Mastrolia
Induced HOXB7 acts to influence phenotype and behavior of marrow mesenchymal progenitors by autocrine and paracrine mechanisms

Francesca Santilli
In vitro model for the evaluation of angiogenic potential of MSCs derived from dental pulp

Mariangela Basile
Chemotactic properties of human amniotic fluid-derived stem cells in mouse calvaria defect model

18.00 *Departure for a guided tour to highline Galleria Vittorio Emanuele II and social dinner*

23 SATURDAY JUNE

08.45 **Attendee registration**

NEURAL STEM CELLS AND THEIR CLINICAL APPLICATION
Chairs: *Gina Lisignoli and Nicola Alessio*

09.00 *Dario Siniscalco*
Possible use of stem cells in autism spectrum disorders: scientific rationale

09.30 *Luciano Conti*
In vitro modelling of human neocortical development using pluripotent stem cells

10.00 *Maurizio Gelati*
Human neural stem cells production for neurodegenerative diseases treatment

10.30 *Letizia Mazzini*
Foetal neural stem cell transplantation in amyotrophic lateral sclerosis: long-term follow-up of a phase 1 clinica trial

11.00 *Coffee break*

11.30 **THREE SELECTED POSTER COMMUNICATIONS BY YOUNG INVESTIGATORS**
Chairs: *Giovanni Di Bernardo and Yvan Torrente*

12.30 **EVALUATION OF ECM CREDITS**

13.00 *Concluding remarks and light lunch*

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Anti-inflammatory and immunomodulatory miRNA cargo of microvesicles obtained from equine amniotic progenitor cells in different time-span of in vitro culture

Anna Lange-Consiglio
Dipartimento di Medicina Veterinaria (DIMEVET) Università degli Studi di Milano

Mesenchymal stromal cells (MSCs) were found to secrete many factors with therapeutic relevance for their anti-oxidants, anti-apoptotic, anti-fibrotic, angiogenic, immunomodulatory and chemiotactic activities. The culture supernatant of MSCs (in our case namely of equine amniotic derived cells) is defined secretome or conditioned medium (CM) and it is composed of soluble and no soluble factors secreted by cells. Soluble factors are represented by cytokines and growth factors, while microvesicles (MVs), that it has recently been demonstrated to be an integral component of cell-to-cell communication during tissue regeneration, represent no soluble factors (Bruno et al., 2009 Journal of the American Society of Nephrology 20, 1053–1067). Our previous data showed that equine amniotic derived CM, administrated in vivo in equine spontaneous tendon lesion, is able to regenerate the injured tissue overlapping the results obtained by using in vivo the cells of origin in the same pathology (Lange-Consiglio et al., 2013 Stem Cells Development 22, 3015–3024). We also studied the amniotic derived MVs and we found that they are involved in downregulation of pro-inflammatory genes in in vitro LPS stressed equine tendon and endometrial cells (Lange-Consiglio et al,. 2016 Stem Cells Development 25, 610–621; Perrini et al,. 2016 Stem Cell Research and Therapy 7, 169). Usually, protocols to produce CM and MVs are different: CM can be collected after 12-96 hours of culturing cells without renewal of tissue culture medium, while MVs are usually collected culturing cells overnight. Future comparative study of regenerative medicine using CM and MVs will be difficult to interpret because there are not information about the quality of secretion of cells and the difference in micro-RNA cargo in MVs isolated after only 1 night of culture or isolated from CM that usually required a longer culture time. In this context, the aim of this relation is to comparatively report the miRNA content in AMCs and their MVs in two time points chosen between 1 night and 4 nights (the longer time of production of CM identified in literature and used in our previous studies) to evaluate the effect of different times of culture on quality of secretome. An enrichment of miRNAs, that have an important role in the immunity and anti-inflammatory response, is found both in cells and MVs at 4 nights of culture compared to 1 night. Simultaneously, an increase of MVs size due to the presence of apoptotic bodies is discovered. It will be interesting to isolate these apoptotic bodies in order to understand how their miRNA content is able to affect the miRNA library. In this way, it would be possible to give a more precise answer to the question if is better the secretoma of 1 night or 4 nights.

Lung mesenchymal stem cells ameliorate elastase-induced damage in an animal model of emphysema

Cappetta D., De Angelis A., Spaziano G., Tartaglione G., Piegari E., Esposito G., Ciuffreda L.P., Liparulo A., Sgambato M., Russo T.P., Rossi F., Berrino L., Urbanek K., D'Agostino B.
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Pulmonary emphysema is a respiratory condition characterized by alveolar destruction that leads to airflow limitation and reduced lung function. The chronic inhalation of irritants attracts inflammatory cells and inflammatory mediators into the lungs, where they impair protease-antiprotease balance thus leading to destruction of alveolar units. Although extensive research, the pathophysiology of emphysema is incompletely understood and effective treatments are still missing. A significant body of evidence have demonstrated that mesenchymal stem cells (MSCs), harvested from adult organs such as bone marrow and adipose tissue, and administered in the damaged tissue, may induce organ repair mainly through paracrine effects. On the other hand, little is known about the biological significance of lung-derived MSCs also because of obvious difficulties to obtain lung biopsies that have limited the studies on these cells. Nonetheless, lung MSCs may be relevant in alveolar homeostasis and repair after injury and may need consideration as a potential tool or target for cell-based therapy that involves other pulmonary cell populations. Thus, the aim of this study was to test the effects of the intratracheal administration of lung-derived GFP-tagged mouse MSCs in a model of elastase-induced emphysema. Flow cytometry analysis showed the expression of surface markers consistent with the profile of cells of mesenchymal origin (CD44, CD73, CD90 and CD105). Immunofluorescence analysis addressing the capacity of MSCs to engraft within the injured tissue, revealed the substantial presence of GFP-positive cells, negative for hematopoietic surface marker CD45, within pulmonary structures ten days after their administration. Histological analysis revealed evident airspace enlargement and obliteration of the alveolar wall in the lungs injected with elastase. These changes were attenuated by the instillation of MSCs. Pulmonary function (static lung compliance) showed an increased stiffness induced by elastase, while morphometric findings (mean linear intercept and tissue/alveolar area) confirmed the severity of alveolar disruption. Contrarily, MSC administration partially restored lung elasticity and alveolar architecture.
Western blotting of elastase-treated lung tissue showed the reduced content of epithelial markers aquaporin 5 and surfactant protein C, consistent with the destructive effect on the alveolar walls. The formation of new epithelium was suggested by the increased expression of aquaporin 5 and surfactant protein C observed in the lungs receiving intratracheal administration of lung MSCs. Indeed, in the MSC-treated group, a significant rate of proliferative cells confirmed the formation of new parenchyma. Interestingly, the analysis of alveolar epithelium revealed a higher proliferative rate of alveolar epithelial type II cells. The hypothesis of a paracrine mechanism orchestrated by MSCs was confirmed by the higher presence of hepatocyte growth factor in the lung parenchyma of mice treated with MSCs. The importance of HGF signaling was supported by the elevated content of its receptor c-Met in the MSC-treated lungs. We report previously unrecognized properties of adult mouse lung-derived MSCs that after local administration boost and coordinate response to damage. Although several aspects of cellular physiology and in vivo behavior related to therapeutic potential of lung-derived MSCs remain to be clarified, the comprehension of epithelial repair-driving mechanisms, as well as the interrelationships between epithelial cells and MSCs, may help to identify targets for pharmacological and/or cell-based interventions for lung diseases.

Human periodontal ligament stem cell differentiation into airway cells: New perspectives for studies on lung diseases

Patruno S.[1,2], Pomilio A.[1,2], Cianci E.[1,2], Lanuti P.[2,3], Bologna G.[2,3], Pierdomenico L.[2,3], Merciaro I.[1], Diomede F.[1], Marchisio M.[2,3], Trubiani O.[1],Romano M.[1,2]

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The availability of primary cells is essential for studies on chronic lung disorders, particularly those of genetic origin with unpredictable genotype/phenotype correlation such as cystic fibrosis (CF). This disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR), coding for a protein with anion channel activity. More than 2000 CFTR mutations are known, however, their impact on disease severity, which affects particularly the respiratory system, and on patient response to therapy is largely unpredictable. Thus, respiratory cells form the patient are paramount for diseases modeling and personalized medicine with the newly introduced CFTR modulator drugs, which require in vitro testing for accurate prediction of the patient's response. We recently isolated stem cells from the human periodontal ligament (hPDLSC). They are defined as ecto-mesenchymal cells, are easy to isolate with minimally invasive procedures and to propagate in vitro with excellent yields and preservation of the original phenotype for a high number of passages. Although their differentiation capability into mesoderm lineages has been largely demonstrated, the observation that hPDLSC express the embryonic stem cell markers Oct4, Sox2, Nanog suggests that they may be able to differentiate into other germinal lines. Here, we tested the hypothesis that under appropriate culturing conditions hPDLSC can differentiate into endoderm derived lineages, such as lung cells. To this end, we exposed hPDLSC from healthy donors to a specific media to enter the definitive endoderm stage, SHH and high concentration of FGF2 to enter into AFE stage and then to a low concentration of BMP4 and minimal concentration of FGF7 and FGF10 to follow the expansion and differentiation of these cells into immature progenitor of lungs.

At the end of the differentiation process, hPDLSC dramatically changed morphology from a fibroblast-like shape to a cuboidal epithelial-like shape. This was accompanied by a considerable increase in the transcripts of lung proximal epithelial markers (Tp63, sox2) and of the type C surfactant protein, typical marker of type II alveolar cells. We also detected a substantial increase in the transcript of CFTR (~ 40 fold) and of the lung specific genes EpCAM (~ 25000 fold), MUC5AC (~ 40000 fold), MUC5B (~ 2.000 fold). The respective proteins increased consistently. The differentiated hPDLSC stained positive for Alcian Blu-PAS visualizing the presence of neutral and acidic mucins. Moreover, they displayed a strong increase in transcellular electrical resistance, which was reduced by ~ 40% by CFTRinh-172, a selective CFTR inhibitor, consistent with the acquisition of a respiratory epithelial phenotype and with the hPDLSC suitability for CF disease modelling and personalized medicine.

In conclusion, we have succeeded at differentiating hPDLSC into airway cells. In aggregate with our previous data showing hPDLSC differentiation into neural cells, these results suggest that hPDLSC may have embryonic-like properties. They also open new perspectives for studies on CF pharmacology and drug discovery as well as for modeling of other chronic lung diseases including chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis.

Myoexosomes cargo triggers muscle regeneration and provides molecular cues for alternative exon skipping theraphy in muscular dystrophy

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Muscular dystrophies are genetic neuromuscular disorder characterized by skeletal muscle degeneration and consequently fibrosis. Duchenne muscular dystrophin is the most common form where mutations on the dystrophin gene located on the X chromosome lead to the depletion of the functional protein. We hypothesized a possible role of exosomes through their protein or/and non-coding RNA content in enhancing muscle regeneration compared to fibrosis in dystrophic environment. This hypothesis has been validated by the discovery in the muscle-derived exosome of the full-length and the Dp71 dystrophin isoform and the muscle-specific miRNAs or, at least, involved in regeneration processes. These pro-myogenic effects have been tested in vivo on the dystrophic murine mouse model (mdx) and in vitro on the satellite cells derived from the mdx mouse. Theses observations highlight the myogenic signature of muscle-derived exosomes and develop new therapeutic strategies for the elucidation of the mechanisms involved in dystrophic muscle progression.

Oxidative stress induces necroptosis in mouse mesoangioblast stem cells

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Nowadays stem cells are extensively used in regenerative medicine, as they show a great potential in restoration of several pathologies such as ischaemic heart disease, stroke, degenerative disorders and so on. However, it is well known that their therapeutic efficacy is compromised not only due to a reduced homing towards the target site, but also for their massive death during the first several days post-transplantation caused by the cytotoxic environment. In fact, the microenvironment within damaged tissues is unfavourable for stem cell survival due to hypoxia, inflammatory mediators and above all oxidative stress, which is particularly detrimental. H2O2 that diffuses freely into and out of cells may play a significant role in inducing death of injected stem cells. The most common types of cell death are represented by apoptosis, autophagy and necrosis. Apoptosis is a process of programmed cell death that occurs in multicellular organisms generally by activating caspases, or enzymes that degrade proteins. Autophagy is an important mechanism of cell self-protection, which helps cells maintain the synthesis and degradation cycles and promotes cell survival through the lysosomal degradation mechanism. Finally, necrosis is known as a fortuitous and regulated means of cell death that is induced by non specific and non physiological stress. The aim of our study was to determine the mechanism of mesoangioblast cell death after an in vitro H2O2 treatment, focusing whether apoptosis, autophagy or necrosis were implied. Mouse mesoangioblasts are vessel associated progenitor stem cells, which are able to differentiate in almost all mesodermal tissues.
FACS analysis with annexinV/sytox green demonstrated that H2O2 induced a dose- and time-dependent decrement in mesoangioblast viability. We have also found an increase in caspases 8, 9 and 3 activity after hydrogen peroxide treatment. To assess whether they were involved in causing cell death, the pan caspase inhibitor Z-VAD-fmk was used to inhibit caspase activity. Neither early apoptosis, nor late apoptosis/necrosis, nor necrosis were reduced, suggesting that the cell death induced by hydrogen peroxide was caspase-independent.
For this reason we tested whether H2O2 is responsible for the autophagic pathway activation. To study autophagy we evaluated the expression of specific markers (e.g., LC3II, beclin1, p62, Atg7, Atg5). H2O2 decreased beclin1, Atg5, Atg7 levels and the ratio LC3II/I, in a dose dependent way. At the same time H2O2 increased p62 protein expression indicating an impaired autophagic flux, also confirmed by the increase of phospho AKT, responsible for the activation of mTOR, a negative regulator of autophagy. According to these data mesoangioblast treatment with hydrogen peroxide seems to not induce nor apoptosis or autophagy. For this reason we hypothesized the activation of necroptosis, which is a specific form of caspase-independent, non-apoptotic or necrotic cell death that is triggered by cell death ligands via a unique downstream signaling pathway. To confirm whether the observed cell death was due to enhanced necroptosis, the proportion of necrotic cells was determined by annexin V/sytox green staining. FACS analysis showed an increase in percentage of both late apoptotic/necrotic and necrotic cells, which were further increased by pretreatment with Z-VAD-fmk.
To investigate the relationship between physiological autophagy and necroptosis cells were treated with H2O2 in the presence of the autophagic inhibitor 3-MA. Annexin V/sytox green staining showed that the inhibition of autophagy by 3-MA significantly enhanced necroptosis in mesoangioblast treated cells. On the contrary, 3-MA had no effect on apoptosis.
In conclusion, our in vitro data indicate that the cytotoxicity of H2O2 in mesoangioblast mainly occurred via the induction of necroptosis, enhanced by both apoptosis and autophagy inhibition.

MICAL2 affects myogenic differentiation

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The dystrophin-associated glycoprotein complex (DGC) serves as a mechanical link between the cytoskeleton and the extracellular matrix of muscle cells. Disassembly of this protein complex renders the sarcolemma vulnerable to contraction-induced injury, leading to progressive fiber damage, membrane leakage and necrosis. DGC interacts with Filamentous-actin (F-Actin), fine regulated by Microtubule Associated Monooxygenase, Calponin And LIM Domain Containing 2 (MICAL2). Indeed, MICAL2 modifies actin subunits and promotes actin filament turnover by severing disaggregation and preventing repolymerization[1]. Interestingly, in a genome profiling study, MICAL2 has been found among a set of ten functionally linked genes involved in muscle degenerations of mdx mice[2].
In this study, we focus on the role of MICAL2 in skeletal, cardiac and smooth muscle differentiation. In particular, we found that murine mesoangioblasts, vessel associated stem cells, express high levels of MICAL2 when differentiated into smooth muscle cells. Adult and embryonic hearts also express MICAL2 that is again upregulated in human induced pluripotent stem cells during differentiation into cardiomyocytes. Similarly, MICAL2 increases during myogenic differentiation of myoblast cell-line and primary skeletal muscle stem cells. Intriguingly, loss of function studies of MICAL2 expression resulted in impaired myogenic differentiation on both stem cell types. On the contrary, gain of function experiments show a positive impact of MICAL2 on skeletal muscle commitment. Finally, in acute and chronic skeletal muscle regeneration MICAL2 appears very highly expressed in regenerating nuclei, indicating its importance in these pathological conditions. Taken together these data demonstrate that modulations of MICAL2 have an impact on muscle differentiations. Further experiments are necessary to discern if the absence of MICAL2 affects also smooth and cardiac muscle differentiation. Moreover, understanding the role of MICAL2 in myogenic commitments and being able to modulate its expression might help the capacity to regenerate of all the muscle cell types.

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Molecular pathogenesis of facioscapulohumeral muscular dystroph

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent neuromuscular disorders and leads to significant lifetime morbidity. Extra-muscular manifestations include retinal vasculopathy, hearing loss, respiratory defects, cardiac involvement, mental retardation and epilepsy.
FSHD is not caused by a classical form of gene mutation resulting in loss or altered protein function. Likewise, it differs from typical muscular dystrophies by the absence of sarcolemma defects. Instead, FSHD is linked to copy number and/or epigenetic alterations affecting the D4Z4 macrosatellite repeat array located in 4q35 causing aberrant overexpression of the D4Z4 encoded double homeobox 4 (DUX4) gene.
DUX4 is a transcription factor that plays an essential role in activating the embryonic genome during the 2- to 8-cell stage of development. As such, it is not normally expressed in most somatic tissues, and importantly it is silent in healthy skeletal muscle. While the exact pathways by which aberrant DUX4 expression leads to muscular dystrophy are currently unknown, ectopic expression of DUX4 in multiple cell lines as well as in skeletal muscle in vivo leads to apoptotic cell death.
Despite several clinical trials, there continues to be no cure or therapeutic option available to FSHD patients. The ability of DUX4 to activate its direct transcriptional targets plays a major role in DUX4-induced muscle toxicity. Accordingly, RNA sequencing found that DUX4-controlled gene expression is the major molecular signature in FSHD skeletal muscle and identified core DUX4 target genes. Thus, preventing the production of DUX4 or inhibiting the ability of DUX4 to activate gene expression have strong therapeutic relevance.
Here, I will summarize our recent results regarding the regulation of DUX4 expression and activity and how they could be targeted for therapeutic purposes.

Paracrine boosting of endogenous mechanisms of cardiac repair and regeneration

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Restoration of the injured heart requires efficient cardioprotection, improvement of cardiac repair, and myocardial renewal following significant loss of cardiomyocytes. Translational research has provided encouraging results, but has not yet fulfilled expectations, possibly because the approaches proposed so far have not addressed the main defects causing progression of heart disease. Yet, the adult mammalian heart retains some regenerative capability, based on cardiac progenitor cell (CPC) activation and resident cardiomyocyte proliferation. Although these mechanisms are widely responsive during cardiogenesis and in the very early stages of post-natal life, they become quiescent and dormant in the adulthood, leaving the heart endowed with an impaired injury response under pathological situations, such as myocardial infarction. Therefore, a working strategy to enhance and restore in full the cardiac endogenous potential for both repair and regeneration will open new frontiers in cardiac medicine.
Recently stem cell biology has been broadly scrutinized in order to define a therapeutic approach. Despite an increasing interest towards the analysis of the cardiovascular differentiation potential of autologous or allogeneic stem cells transplanted into the injured heart, particular attention has been lately addressed to their paracrine modulatory influence instead. Indeed, the so called "paracrine effect" has been proposed as a promising strategy to boost endogenous mechanisms of repair and regeneration from within the cardiac tissue. This has led to a significant paradigm shift: from exploring the stem cell genome and the direct differentiation potential to analysing the stem cell "secretome", as the whole of growth factors and chemo-attractant molecules produced by the stem cells via paracrine secretion.
Hence, the scientific community is now focusing on identifying the ideal stem cell population endowed with the most effective cardioactive secretome and in such scenario human fetal but not-embryonic progenitors from the amniotic fluid might represent an appealing source.

Induced pluripotent stem cells technology: toward precision medicine in cardiology

Massimiliano Gnecchi

Can we distinguish fetal, adult and iPS-derived MSCs?

Lorenza Lazzari

Unfolding the stem cell world: a path among the use of molecules and physical energies

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Stem cells are widely defined as cells with no specific distinct features which are able to self renew and differentiate under specific environmental stimuli. Stem cell behaviour is further complicated by the presence of a stem cell niche, which mediates the interaction between stem cells and the surrounding milieu. Defining specific chemical or physical stimuli, acting as modulators of stem cell behaviour could offer novel tools for regenerative medicine and clinical application. Conditioned media have been widely used to commit stem cells toward specific phenotypes defining the appearance of tissue specific elements exhibiting defined cellular functions. In particular, during the last years we used a mixture of hyaluronic, together with butyric and retinoic acid to commit mouse embryonic and human mesenchymal stem cells from different sources, as bone marrow, placenta, dental pulp and amniotic fluid toward the cardiovascular phenotype[1]. Recently the same cocktail, together with the circadian related hormone melatonin, was successfully used by our group to obtain an osteogenic phenotype from dental pulp derived stem cells[2]. Concerning melatonin we recently demonstrated a role of this hormone and of vitamin D in counteracting the appearance of an adipogenic fate in adipose derived stem cells, offering novel strategies in counteracting fat depot and thus obesity. Besides drugs, also physical energies are able to affect stem cell behaviour[3]. In 2005, we used extremely low-frequency magnetic fields to induce a molecular pattern of cardiogenesis and a high yield of cardiac beating clusters in mouse embryonic stem cells[4]. Recently, we demonstrated that radiofrequency waves emitted by the Radio electric asymmetric conveyer (REAC) were able to induce the appearance of cardiogenic, myogenic and neurogenic phenotypes both in murine embryonic and in human adipose derived stem cells[5]. REAC was also able to induce a direct reprogramming of human adult fibroblasts toward cardiac, skeletal muscle and neuronal phenotypes[6]. This effect was related to the activity of NADPH oxidase, an enzyme which controls the production of ROS inside the cells. So REAC could act as an hormetic effector, as for example hypoxia or other types of stressors, which at low doses stimulate cells to react by self repairing strategies. The physical energy emitted by REAC was also able to induce the neurogenic differentiation of the pheocromocitoma cells PC12, by inducing an NGF-mediated paracrine circuitry[7]. Based upon our results and also some clinical studies demonstrating the capability of REAC to act as a regenerative inducer, we could consider the chance to use a physical energy able to stimulate the patient's own self reparative potential. Nevertheless there are many events especially at the molecular level which still remain to be clarified, and need further experimental dissection. Taken together, our results define novel molecules and novel applications, and unfold innovative tool for in vitro differentiation of stem cells and future clinical applications.

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Therapeutic plasticity of neural stem cells

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Recent evidence consistently challenges the sole and limited view that neural stem/precursor cells (NPCs) may protect the central nervous system (CNS) from inflammatory damage leading to neurodegeneration exclusively throughout cell replacement. As a matter of fact, NPC transplantation may also promote CNS repair via intrinsic neuroprotective bystander capacities, mainly exerted by undifferentiated stem cells releasing, at the site of tissue damage, a milieu of neuroprotective molecules whose in situ release is temporally and spatially orchestrated by environmental needs. This milieu contains molecules (e.g. immunomodulatory substances, neurotrophic growth factors and stem cell regulators), which are constitutively expressed by NPCs for maintaining tissue homeostasis either both during development and adult life. The intrinsic nature (pleiotropism and redundancy) of these molecules as well as their 'constitutive' characteristics, may also reconcile data showing that other sources of somatic stem cells (e.g. mesenchymal stem cells), with very low capabilities of neural (trans) differentiation, may efficiently promote CNS repair. Thus, cell plasticity can also be viewed as the capacity of somatic stem cells to adapt their fate and function(s) to specific environmental needs occurring as a result of different pathological conditions (therapeutic plasticity). The challenging ability of transplanted NPCs to protect the brain from several types of injuries using different and/or articulated bystander strategies is of pivotal importance for the future of stem cell based therapeutic approaches.

Induced HOXB7 acts to influence phenotype and behavior of marrow mesenchymal progenitors by autocrine and paracrine mechanisms

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Identified 50 years ago, mesenchymal stromal/stem cells (MSC) were immediately generating a relevant interest in the scientific community for their differentiation plasticity and the hematopoietic supportive function. Nowadays, BM MSC-like populations has been described in a variety of sources including: adipose tissue, skin, cord-blood, warthon jelly, placenta, amniotic fluid, liver, bile ducts, lung, teeth and more. However, bone marrow (BM) is considered the main source of MSC used for tissue regeneration, in particular in the orthopedic field. The particular interest around bone defects, which are often associated with a relevant morbidity has led to a clinical implementation of bone regeneration approaches. In a recent study, we have revealed the key role of homeobox protein HOXB7 in MSC proliferation and osteogenic differentiation, thanks to an autocrine loop of basic fibroblast growth factor (bFGF).

Thus, we asked whether the gene-induced expression of HOXB7 in MSC could enhance their stromal function as "living bioreactors" capable to impact also on non-modified MSC by the secretion of mitogens like bFGF. We challenged the conditioned medium (CM) of HOXB7 modified MSC with non-modified MSC, observing variations in their cell growth and FACS-based physical parameters (SSC/FSC) compared to the control, only when we used a 3:1 ratio between CM producer and target MSC. We have then co-cultured MSC-WT with MSC-HOXB7 showing a higher proliferation compared to the co-culture of MSC-WT with MSC-GFP. After 10 days of co-culture the 1:1 ratio of GFP positive/GFP negative cells was maintained for the combination MSC-WT+MSC-HOXB7 suggesting that both populations contribute to the increased growth rate. The co-culture with MSC-HOXB7 was interestingly associated with a significant change in MSC-WT morphological parameters: smaller and less complex cells than the controls. Interestingly, the effect was reversible and, after one passage from sorting, modifications acquired during co-culture were lost. When differentiated in vitro, the co-culture MSC-WT+MSC-HOXB7 revealed a significant increase of skeletal commitment compared to MSC-WT+MSC-GFP and MSC-HOXB7 or MSC-WT taken individually. These findings originated an unexplored approach to enhance pivotal MSC features such as proliferation and skeletal regeneration further providing insights on human stem cells aging. Their possible clinical implications led us to verify the in vivo impact of MSC-HOXB7 in a xenogenic model of bone regeneration confirming the importance of MSC-HOXB7 in supporting bone formation.

Gene induced HOXB7 expression in MSC could generate novel therapeutic options in bone regenerative potential via a still unexplored by-stander mechanism on modified MSC themselves and on neighbor cells.

This work was made possible in parts by grants from the European Commission FP7/2007-2013 (grant no. 241879) REBORNE Project and H2020 Programme ORTHOUNION Project (grant no 733288), Regione Emilia Romagna: Programma di Ricerca Regione-Università 2010–2012—Strategic Program "Regenerative Medicine of Cartilage and Bone (grant no. PRUa1RI-2012-007), Progetto Dipartimenti Eccellenti MIUR 2017 and Fondazione Guido Berlucchi.

In vitro model for the evaluation of angiogenic potential of MSCs derived from dental pulp

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Mesenchymal stem cells (MSCs) are adult multipotent cells distributed in different anatomic sites where they are thought to play a key role in tissues homeostasis. Several data demonstrate that MSCs could be both efficiently propagated in vitro and induced to differentiate into cells of mesenchymal lineage in the appropriate culture conditions. However, several aspects remain to be elucidated, including the degree of potency belonging to MSCs from different tissues, and the best culture conditions suitable to reach the translational objective.

In our study, we aimed to explore the phenotypic and functional characteristics of human MSCs isolated from dental pulp (DPSCs), and in particular, we investigated the ability of DPSCs to modulate in vitro angiogenesis. For this purpose, we performed co-culture tubule-formation assays using human endothelial cells (HUVECs) in combination with DPSCs.

DPSCs analysed by FACS within two weeks since cell isolation expressed high levels of stem cell markers, including CD90, CD105 and CD44. When used in the co-culture model, DPSCs were able to interact with HUVECs and, significantly, they stabilize HUVEC tubules generating stratified cell structures, in which DPSCs wrap HUVEC tubules. The resulting angiogenic net remained stable for several days; otherwise, in absence of DPSCs, HUVECs underwent apoptotic cell death after 24h. Expression of N-cadherin on both cell types was responsible for heterotypic interaction. However, the pro-angiogenic effect was evident only when DPSCs have previously been cultured in complete endothelial growth medium (EGM-2). Accordingly, FACS and western blot analyses revealed that EGM-2 stimulated a significant phenotypic switch in DPSCs, with an increased expression of pericyte markers, including NG-2, respect to DPSCs cultured in standard conditions. The low expression of endothelial markers in EGM-2-conditioned DPSCs further supported their role as perivascular cells in the angiogenic model.

In physiological condition, pericytes envelop the surface of the vascular tube contributing in stabilization of the vessel, and supporting wound repair. Thus, the further characterization of factors needed for pericyte differentiation, could render DPSCs a new useful therapeutic tool in angiogenic defective clinical situations.

Chemotactic properties of human amniotic fluid-derived stem cells in mouse calvaria defect model

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Currently, treatment of large bone defect is based on autologous or allogenic bone grafts that still have limitations and issues. Bone tissue engineering with cell transplantation could provide an alternative way to stimulate bone repair. In the past few years, numerous studies have been conducted to investigate the osteogenic potential of human amniotic fluid-derived stem cells (hAFSCs) in the repair of bone defects. The absence of ethic controversy and risk of teratoma formation makes hAFSCs particularly interesting in the field of regenerative medicine. Despite this, there is ample discussion in literature about the effects and role of hAFSCs in bone tissue repair, since their fate in vivo in the repair of large bone defects is still unknown at present [1, 2].
The aim of this study was to investigate the role of pre-differentiated hAFSCs in the repair of critical-size bone defects in calvaria mouse model. For this purpose, we transduced hAFSCs with Ub cherry lentivirus and used a recipient transgenic mouse model carrying GFP fluorescent reporter. Thanks to these systems, we were able to follow the fate of hAFSCs transplanted in vivo into Healos® (collagen-hydroxyapatite scaffold) construct and to distinguish donor and host cells at the implant site.
Our results showed that viral transduction of hAFSCs produce a population permanently labeled with cherry red fluorescent protein useful to track the fate of these cells in vivo directly at the site of transplantation. Flow cytometry analysis demonstrated a lower expression of mesenchymal markers on the surface of pre-differentiated hAFSCs compared to undifferentiated cells, suggesting a partial commitment to osteogenic lineage, useful for in vivo transplantation studies. Moreover, we observed that hAFSCs are able to attract mouse bone marrow stromal cells (mBMSCs) in vitro, suggesting a possible chemotactic role of their releasing soluble factors. In accordance with previous results reported by other authors, we showed that cherry red fluorescent hAFSCs, once transplanted in vivo, are not present at the implant site after 3 and 6 weeks. Instead, the presence of a greater number of GFP positive cells in the scaffold at the same time-intervals, compared with implants using Healos® and mBMSCs, indicates that hAFSCs are able to recruit host cells during the repair process. These observations help clarify the role of hAFSCs in bone tissue repair. Further studies will be needed to determine whether the host cells that are recruited are able to form new bone.

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Possible use of stem cells in autism spectrum disorders: scientific rationale

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Autism spectrum disorder (ASD) is characterized as possessing persistent deficits in social communication and interaction, restricted, repetitive patterns of behavior, interests, or activities. The core symptoms of ASDs concern the cognitive, emotional, and neurobehavioural domains.
These disorders are complex, and severe heterogeneous neurodevelopmental pathologies with well characterized immune system abnormalities and inflammatory conditions. Nowadays, cell therapies have been proposed and applied to ASD. Several stem cell types have been proposed in restoring immune imbalance. Stem cells possess the immunological properties which make them promising candidates in ASD treatment. This review will focus on animal models and current clinical trials on the use of stem cells in treating autism.

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In vitro modelling of human neocortical development using pluripotent stem cells

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The human brain is composed by many distinct neuronal subtypes spatially and functionally organized that originate during development by an elaborate sequence of events that, if altered, can lead to defined pathological conditions. Until recently, efforts to study the development and function of the human cerebral cortex in health and disease have been limited by the availability of appropriate in vitro human model systems. Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (iPSCs), provided opportunities to generate human neural progenitors and neuronal populations cells to the understanding of human brain development and to study the human diseases and regenerative medicine dynamically. Here we discuss the optimization of a robust, multistep process for the generation of large numbers of homogeneous defined populations of MGE and cortical progenitors. We discuss also their further neuronal terminal differentiation to acquire properties of Parvalbumin- and Somatostatin-positive cortical GABAergic interneurons and different cortical glutamatergic neurons. These cultures provide a versatile platform for allowing a detailed molecular and functional interrogation of human cortical development, function and disease.

Human neural stem cells for neurodegenerative diseases treatment

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Amyotrophic Lateral Sclerosis (ALS) is an incurable disease that targets motor neurons (MNs) in the primary motor cortex, brainstem and spinal cord. The mechanisms of the inherent neurodegenerative process are still largely unknown Human neural stem cells (hNSCs) represent a potential therapeutic option for ALS. Their inherent functional plasticity allows hNSCs to carry out a plethora of healing actions, spanning the replacement of dead cells, immunomodulation, anti-inflammatory, trophic, homeostatic, scavenging and toxicity-blunting effects. Following non clinical and pre-clinical studies, we established a cGMP protocolthat allows the establishment of continuous, stable, plentiful and standardized hNSCs lines. Indeed, those hNSCs have been used in a Phase I clinical trial (EudraCT 2009-014484-39) in which 18 ALS patients received multiple grafts of these cells. The trial was completed successfully, follow up exceeding four years landmark. hNSCs induced no severe adverse events and were associated with a transitory improved neurological function in 50% of patients. We are now planning a Phase II clinical trial on ALS and a Phase I trial on multiple sclerosis patients is currently ongoing.

Foetal neural stem cell transplantation in amyotrophic lateral sclerosis: long-term follow-up of a phase 1 clinica trial

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Background: Stem-cell-based therapies may represent a new strategy in ALS clinical research. The main objective of this Phase 1 clinical study was to assess the feasibility and toxicity of micro- transplanting GMP-grade, stable and continuous human neural stem cell lines (hNSCs) into the anterior horns of ALS patients' spinal cord. The secondary endpoint was the assessment of potential neurological effects.

Methods: Eighteen patients with definite ALS received either three unilateral (n=3) or three plus three bilateral (n=6) microinjections of hNSCs into the lumbar or cervical spinal cord tracts. All patients were routinely monitored prior and after transplantation by clinical, psychological, neuroradiological and neurophysiological assessment.

Findings: None of the patients manifested severe adverse events or increased progression of the disease as a consequence of the treatment for up to 60 months after surgery. Eleven patients died and 2 patients underwent tracheotomy 7-51 months after surgery due to the natural history of the disease. A significant decrease of ALS-FRS-R progression ($p<0{\cdot}0136$) beginning within the first month post-surgery up to four months following hNSCs transplantation was detected.

Interpretation: This trial shows that transplantation into the spinal cord of ALS patients of hNSC lines is a safe procedure which causes no major short- or long term deleterious effects. This study is the first example of medical transplantation of a highly standardized cell drug product that can be reproducibly and stably expanded ex vivo, with cells from the very same donor being available for the whole study, as well as across future trials. This provides unquestionable benefits related to enhancing both intra- and inter-study reproducibility and homogeneity. In view of the potential, perspective therapeutic effects elicited by hNSCs, our observations lend to the future implementation of phase II clinical studies, investigating the effects of increasing cell dosages in larger cohorts of ALS patients.

Obesity alters the functions of visceral mesenchymal stromal cells: a contribute to chronic inflammation

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In humans and other mammals, there are two main types of white adipose tissue (WAT): subcutaneous (SC), which is present beneath the skin and visceral (VS) that is localized around internal organs. The two types of WATs are different in their pathophysiological properties including insulin sensitivity, adipokine secretion, lipolysis and development of inflammation[1]. There are several findings showing that in obese people the presence of high level of VS is more dangerous for health than SC[2].

Obesity is a medical condition in which excess body fat accumulated to the extent that it may have a negative effect on health. In particular, obesity is associated with chronic inflammation[2]. It is well known that, in overweight and obese people, the level of various circulating cytokines, hormones, and other signaling molecules are dysregulated due to presence of an inflammatory status[3]. This may affect the functions of several organs and tissues, including the stem cell niches, which are specific tissue regions that house stem cells and control their self-renewal and lineage production by modulating the concentration of signaling molecules, such as hormones, cytokines, and growth factors.

Within the adipose tissue there are Mesenchymal stromal cells (MSCs). These cells are heterogeneous because they are composed of distinct cell populations: stem cells, committed progenitors, and mature cells. Stem cells of MSCs can give origin to progeny having a mesodermal phenotype such as osteocytes, chondrocytes, adipocytes and muscle cells[4].

For these reasons, the objective of the present study was that to evaluate how obesity can affect functions MSCs deriving from different sources. We compared in vitro biological properties of MSCs isolated from subcutaneous fat (SC-MSCs), visceral fat (VS-MSCs) and Bone Marrow (BM-MSCs) both in normal weight and obese mice.

Preliminary data demonstrated that SC-MSCs, irrespective of obesity or normal weight conditions, showed a stronger proliferation rate and a higher clonogenic potential compared with VS-MSCs and BM-MSCs. No clear differences in apoptosis were detected. Of great interest, in obese mice the VS-MSCs evidenced a significant increase in the percentage of senescent cells with respect to corresponding cells in normal weight mice. These results suggest that senescence of VS-MSCs may contribute to inflammation phenotype observed in VS, since senescent cells secrete many factors, collectively called SASP (senescence-associated secretory phenotype). SASP contribute to cellular proliferative arrest through autocrine/paracrine pathways but it contains dozens of pro-inflammatory citokines that may sustain inflammation in obese animals.

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Senescence of parenchymal and stem cells in a mouse model of liver cirrhosis

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Cellular senescence is the permanent arrest of cell cycle, related to aging and aging-associate diseases. It plays a crucial role in some organs development and it is a biological mechanism that limit the regenerative potential of stem cells, which may become cancerous. Senescence cells show marked changes in their transcriptomes and therefore begin to secrete a wide range of factors, including cytokines and chemokines. This secretome is known as "senescence-associated secretory phenotype" (SASP) which is able to modify contiguous cell behavior (inducing proliferation or senescence) and amplify the inflammatory process.
Inflammation, the organismal response to an endogenous or exogenous insult, has the purpose of transporting defensive materials in damaged tissue compartment.
Cirrhosis is a disease caused by a chronic inflammation of the liver, which responds to a lesion or to a pathological process by destroying parenchymal cells and replacing them with inter-tissue scars. As a result, the liver gradually loses architecture and functions, since it can no longer regenerate its damaged cells. Currently, there are no effective therapy protocols for cirrhosis and medical treatment is limited to slow its evolution. Assuming that there is chronic inflammation in cirrhosis, we focused our attention on molecules that are secreted by parenchymal cells in response to inflammation stimuli. In particular, we investigated those that can impair cell functions by triggering senescence phenomena. In this setting, Alessio et al identified some pro-senescent secreted molecules that are released following stress stimuli. Three of these proteins (IGFBP-4, IGFBP-5 and IGFBP-7) appear to be associated to senescence and their release to genotoxic stress, including pro-inflammatory events. They belong to insulin-like growth factor binding protein (IGFBP) family based on structural similarities and on functional abilities to bind insulin-like growth factors (IGFs) with high affinities. They are found in biological fluids and regulate the IGF pathways. Considering that in recent years IGFBPs showed more complex activities that are either IGF-dependent or IGF-independent, we decided to investigate the relationship between reactive oxygen species (ROS)-damage and IGFBPs release by senescent cells. In fact, in cirrhotic patients there are well described alteration in ROS serum levels along with chronic inflammation.
In a preliminary study, we used mesenchymal stromal cells (MSCs) to evaluate IGFBPs secretion following ROS-stress that is strictly associated with inflammation process. MSCs are a heterogeneous population, containing a stem cell subpopulation, which can differentiate into mesodermal lineage cells. Our results suggested that events increasing ROS production and causing inflammation may induce cellular senescence along wıth the release of gerontogen proteins (IGFBPs). Subsequently we performed analysis on cirrhotic patients to evaluate if there are any correlations between IGFBPs levels and progression of the disease. Adult cirrhotic subjects (ACS) sera and adult healthy subjects sera (AHS) were collected and IGFBP-4, IGFBP-5 and IGFBP-7 expression was evaluated by western blot.
Both in pooled or single sera we observed an increased expression of the IGFBPs proteins in cirrhotic subjects compared to healthy subjects sera. Therefore, we assessed if this was derived from the damaged liver's stromal cells or from stromal component of bone marrow. To this end we used a mouse model of cirrhosis. In these animals, after a liver damage treatment, we collected liver sections, bone marrow and sera. In cirrhotic animals we observed an increase of circulating IGFBPs, mainly in bone marrow samples but also in liver samples. These data suggest that liver damage may induce IGFBP release that enter into blood flow and hence reach bone marrow triggering the senescence of stromal cells along with further release of IGFBPs (vicious circle).

In vitro characterization of the calvarial suture skeletogenic stem cell niche in nonsyndromic craniosynostosis

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Nonsyndromic craniosynostosis (NCS) is a congenital defect due to the premature fusion of skull sutures. The suture mesenchyme houses a skull-specific stem cell niche, which is plausibly impaired in NCS fused suture sites. To test this hypothesis, we characterized the stem cell niche of open and fused sutures of NCS patients.

Lineage-specific markers were analyzed, by qPCR and immunofluorescence, in suture tissues and in calvarial mesenchymal stem cells (CMSC). MSC from alternative tissues served as controls.

We analyzed the localization of THY1 (skeletal stemness-marker), GLI1 (putative calvarial stemness-marker) and AXIN2 (mesenchymal cell fate determinant) in suture tissue sections: AXIN2 resulted mainly expressed at the endosteal ossified side, while THY1 and GLI1 were primarily expressed within the trabeculae, enriched with proliferating cells.

Both NCS suture tissues and CMSC isolated thereof expressed reduced levels of TEK and ENPEP (bone marrow stem cells differentiation markers) compared with controls. AXIN2 levels were higher in open suture-derived CMSC than in fused suture cells and in controls. Upon in vitro osteogenic induction, the expression of THY1 and GLI1 decreased, whereas AXIN2 levels increased, in both open- and fused- suture derived CMSC.

CMSCs isolated from both fused and unfused sutures shared the same marker expression profile, indicating that explant cultures allowed selecting comparable cell populations, THY1+/GLI1+ representing the stem cell population within the human calvarial niche. Our data seem to suggest that in NCS the in vivo tissue microenvironment may cause the enhanced osteogenic differentiation of suture MSCs leading to premature suture closure.

Fibrosis rescue improves cardiac function in dystrophin-deficient mice and Duchenne patient-specific cardiomyocytes by immunoproteasome modulation

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Patients affected by Duchenne Muscular Dystrophy (DMD) develops a progressive dilated cardiomyophathy (CM) characterized by inflammatory cell infiltration, necrosis and cardiac fibrosis. Standard treatments consider the use of β-blockers and angiotensin-converting enzyme inhibitors that are symptomatic and unspecific towards DMD disease. Medications that target the cardiac fibrosis are in early stages of development. Here, we demonstrated immunoproteosome (IP) dysregulation in affected heart of mdx (murine animal model of DMD) and cardiomyocytes derived from induced pluripotent stem cells (iPSCs) of DMD patients. More interestingly, IP inhibition ameliorates cardiomyopathy of mdx mouse and reduces the development of cardiac fibrosis. Our finding of a cardioprotective function of IP expression suggests its modulation among novel treatments to be tested in future clinical trials to rescue dilated CM of DMD.

Identification of two novel subpopulations of skeletal muscle stem cells with different kinetic of activation

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Previous work from our laboratory in the mdx mouse model of Duchenne muscular dystrophy (DMD) demonstrated that satellite cells (SCs), the stem cells responsible for muscle regeneration, progressively acquire fibrotic features during the progression of the disease. In the process of characterising this aberrant behaviour we serendipitously discovered that skeletal muscle SCs might be separated into two distinct subpopulations, on the basis of the expression of a specific mesenchymal stem cell "signature". Crucially, this separation does not correlate with the acquisition of fibroblast features; rather it separates the pool of SCs in two subpopulations, both maintaining the myogenic properties in healthy muscles. Although accumulating evidence suggests that the pool of muscle stem cells is far to be homogeneous, this is the first documentation of the presence of two populations, which are numerically comparable in healthy muscle. These two newly identified subpopulations presented a strikingly different response in terms of kinetic of activation and differentiation during the regenerative process induced by acute muscle injury. Moreover, the mesenchymal-marker+ve subpopulation of SCs was able to give rise to mesenchymal-marker-ve SCs and therefore appeared to be upstream in the lineage hierarchy. Importantly, the specific aberrancies of muscular homeostasis occurring in the dystrophic muscle of mdx mice led to an alteration in the ratio of these two subpopulations, and to the partial loss of the mesenchymal-marker+ve population. These observations expand the concept of heterogeneity of muscle stem cells and provide an important step towards the comprehension of the mechanisms that guide normal vs. pathological skeletal muscle regeneration.

Induced pluripotent stem cell-derived mesodermal progenitors offer a translatable cell source to treat muscular dystrophies

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Muscular dystrophies are a group of neuromuscular disorders primarily characterized by progressive muscle weakness. As both cardiac and skeletal muscle are affected, patients would benefit from stem cells/progenitors that has the ability to regenerate both tissues. Recently, our group has successfully developed a protocol to generate induced PSC–derived mesodermal progenitors (MiPs) that have the ability to regenerate both cardiac and skeletal muscle[1]. Although promising, several issues remain to be addressed. Firstly, the MiPs lack clinical potential as they are derived through a serum-containing embryoid body-induced differentiation protocol. Therefore, we aimed to derive a new pool of mesodermal progenitors through a fully chemical-defined monolayer approach, thereby enhancing their clinical translatability. Secondly, functional improvement of both tissues was only moderate. To address this problem, focus was put on valproic acid (VPA), a small molecule that can serve as a histone deacetylase inhibitor. VPA has already been implemented in both myogenic capacity of adult stem cells and early cardiac specification[2,3]. Furthermore, a connection has been found between VPA and Notch1 pathway, a signaling pathway found in important in both myogenic and cardiac differentiation[4,5]. Nevertheless, little is known about its effect on early myogenic differentiation. Therefore, we will study the effect of VPA the myogenic capacity of the MiPs and whether this effect is mediated through the Notch1 signaling pathway.

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MELATONIN AND VITAMIN D ORCHESTRATE THE OSTEOGENIC FATE TRHOUGH EPIGENETIC MECHANISMS

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Fat tissue represent an important source of stem cells, the so called "Adipose derived stem cells" (ADSCs), which can differentiate towards different phenotype under external stimuli, retaining a sort of favorite pre-commitment toward the adipogenic fate. Melatonin, a natural hormone that regulate many physiological processes[1], including circadian rhythms, is a molecule able to promote osteoblast maturation in vitro and to prevent bone loss in vivo[2]. Other authors also demonstrated a role of melatonin in negatively regulating glucose uptake and adipocyte metabolism[3]. In this regard, we have previously shown that melatonin in combination with vitamin D was able to counteract the appearance of an adipogenic phenotype in the presence of an adipogenic conditioned medium. In the present study, we aimed at evaluating the specific phenotype elicited by melatonin and vitamin based medium, considering also the involvement of epigenetic regulating genes. Histone deacetylases 1 and Sirtuins (Silent Information Regulator) 1 and 2, belongs to the same superfamily of enzyme able to remodel chromatin structure, thus controlling gene expression[4,5], but Sirtuins 1 and 2 seem particularly related to metabolic homeostasis, aging and embryonic stem cell differentiation[6,7,8]. Both of them are described to be activated after melatonin stimulation, as for example during the dark in response to fasting but not specifically in ADSCs[9].

To evaluate the cell differentiation and the epigenetic regulation of these processes, ADSCs were cultured in a specific adipogenic conditioned media (ADM), in the presence of 0.01 M melatonin (Melatonin+DM) alone or in combination with 10-6 Vitamin D (Melatonin+VitaminD+DM). Cells used as a control were maintained in a basic growing medium. The expression of specific osteogenic related genes Bmp2, Osteocalcin and Stanniocalcin, HDACs 1 and SIRT1 and 2 was evaluated at different time points. Alizarin Red assay was performed to evaluate the mineralization after 21 days of differentiation. Our results show that Melatonin and Vitamin D are able to modulate ADSCs commitment towards osteogenic phenotype trough inhibition of adipogenesis and the upregulation of HDAC, Sirtuins 1 and 2.

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The Alizarin Red shows the calcium/fosfate cytoplasmatic accumulation in cells treated with melatonin together with Vitamin D, as compared to undifferentiated cells, despite the presence of the adipogenic medium. Our findings unfold an epigenetic regulation of melatonin in stem cell differentiation inducing HDAC gene and protein expression and could open up novel strategies for future therapeutic balancing stem cell fate toward adipogenic or osteogenic phenotype.

Blocking CD248 molecule, in perivascular stromal cells of Systemic Sclerosis patients, strongly inhibits their differentiation toward myofibroblasts and proliferation. A new potential target for antifibrotic therapy

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Objective. Fibrosis may be considered the hallmark of Systemic Sclerosis (SSc), the end-stage triggered by different pathological events. Transforming growth factor-beta (TGFβ) and platelet-derived growth factor-BB (PDGF-BB) are profibrotic molecules, modulating respectively myofibroblast differentiation and proliferation. There are evidences, linking CD248 with these two molecules, both highly expressed in SSc patients, and suggesting that CD248 may be a therapeutic target for several diseases. The aim of this work was to evaluate the expression of CD248 in SSc skin and its ability to modulate SSc fibrotic process.

Methods: After ethical approval, skin biopsies were collected from 20 SSc-patients and 10 healthy control (HC). CD248 expression was investigated in the skin, and in bone marrow mesenchymal stem cells (MSCs) treated with TGFβ or PDGF-BB, by immunofluorescence, qRT-PCR and western-blot. Finally, in SSc-MSCs, CD248 gene was silenced by siRNA.

Results: An increased expression of CD248 was found in ECs and perivascular stromal cells of SSc-skin. In SSc-MSCs the levels of CD248 and αSMA expression were significantly higher than in HC-MSCs. In both SSc- and HC-MSCs, PDGF-BB induced an increased expression of Ki67 when compared with UT-cells but was unable to modulate CD248 levels. After CD248 silencing, both TGFβ and PDGF-BB signaling were inhibited in SSc-MSCs.

Conclusion. CD248 over-expression may play an important role in fibrotic process by modulating the molecular target leading to perivascular cells differentiation toward myofibroblast, and interfering with its expression, might open new therapeutic strategy to inhibit myofibroblast generation during SSc.

Endothelial committed periodontal ligament stem cells: an in vitro model to correlate periodontal and cardiovascular diseases

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The discovery that the oral stem cells are capable of differentiating into endothelial cells raises the exciting possibility that these cells can be an autologous source for vascular networks and can represent a model for the study of endothelial disease. Porphyromonas gingivalis, has been identified as one of the major pathogens involved in the progression of periodontal disease, causing chronic inflammation in oral cavity and affect the systemic human health in particular the cardiovascular system is related. Several studies have demonstrate that periodontitis are associated with cardiovascular disease. The purpose of this study was to induce periodontal ligaments stem cells (hPDLSCs) toward endothelial differentiation, evaluation of the molecular signaling pathway involved in the response to the LPS-G, and thefunctional response evaluation of the living construct constituted by porcine decellularized valve/e-hPDLSCs treated with LPS from Prophyromonas Gingivalis (LPS-G). For ex vivo endothelial differentiation, hPDLSCs were cultured with endothelial growth medium (EGM-2MV) supplemented with vascular endothelial growth factor (VEGF). Uninduced and induced cells were treated with 5ug/ml of LPS-G for 24h. Cell growth was evaluated trough MTT test and proinflammatory cytokines were evaluated trough a Quantybody array (RayBiotech), Reactive Oxygen Species (ROS) generation were also evaluated. Morphological analyses were carried out using ligh, scanning electron and confocal laser microscopy systems. Obtained results showed that 5μg/ml LPS-G stimulus provokes: a slowdown of cell growth starting from 24hr and the release of IL6, IL8, and MCP1 molecules. Signaling network analyzed showed the activation of TLR4/ NFkB/ERK1/2/p-ERK1/2 signaling mediated by MyD88 in LPS-G stimulated e-hPDLSCs, moreover a time course put in evidence a nuclear traslocation of ERK1/2 and p-ERK1/2 in differentiated samples. Following, the ability of e-hPDLSCs to expand and colonize the decellularized porcine heart valves was appraised at ultrastructural level. Considering that, ROS play an important role in the progression and development of cardiovascular disease (CVD), in LPS-G living construct model e-hPDLSCs/ decellularized porcine heart valves (dPHV), ROS production was assessed. Time lapse experiments evidenced that LPS-G provokes in e-hPDLSCs a rapid and sustained increase in ROS generation, negligible on undifferentiated cells. From obtained data, by multiparametric analyses, a reasonable conclusion may be that the inflammation process activated by LPS-G can affect endothelial cells and could represent in vivo a possible pathological and predictor state of CVD

Conditioned medium from amniotic membrane-derived cells protects striatal degeneration and ameliorates motor deficits in the R6/2 mouse model of Huntington's disease

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Huntington's disease (HD) is a fatal, incurable autosomal dominant neurodegenerative disorder, with an incidence of 5 10 in 100,000 individuals worldwide (Huntington's disease collaborative research group, 1993). HD is caused by an increase in CAG repeats in the HTT gene, leading to an unstable expansion of polyglutamine (polyQ) tract within the resulting mutated HTT protein (mHTT) (MacDonald M, 1993).

The toxic functions of mutant Htt (mHtt) include aberrant activation of various signaling pathways, altered gene expression, and neurodegeneration (Kim et al., 2001; Zuccato et al., 2010). Recent studies suggest that mHtt may also induce the proliferation and activation of microglia (Crotti et al., 2014). Persistent microglia activation in HD may exacerbate neurodegeneration. Indeed, the activation of microglia predicted onset of symptoms of HD and pre- symptomatic HD patients, who also display elevated levels of inflammatory cytokines, thus supporting defect in the neuro-immune signaling pathways (Politis et al., 2015). These findings indicate that microglia activation may represent a critical juncture in the progression and amplification of HD pathology that, if understood, could support the development of anti-inflammatory based patient treatment options.

MSC from the amniotic membrane (hAMSC) and their conditioned medium (CM-hAMSC) possess immunomodulatory properties in vitro (Magatti et al., 2008, 2015, 2016) and in vivo in animal models of immune-based disorders (Parolini et al.,2014).

Of particular interest is a recent finding that hAMSC and CM-hAMSC induced comparable protection in an in vitro model of traumatic brain injury (TBI), and specifically promote neuronal rescue, M2 microglia polarization, and induce trophic factors indicating that the cross-talk between hAMSC and the damaged brain is not crucial for hAMSC to release bioactive factors (Pischiutta et al. 2016). At present, there are a few options to treat the symptoms of HD and no therapy to slow the neurodegenerative process.

In this study, we sought to determine if CM-hAMSC could alleviate neurological deficits and brain pathology in a highly utilized model system, the R6/2 mouse.

Material and Method Transgenic mice were treated with CM-hAMSC daily starting from 5 weeks of age until euthanasia. Neurological evaluation (paw clasping, rotarod performance, locomotor activity in an open field) was performed twice a week. After transcardial perfusion, histological and immunohistochemical studies were performed.

Results We found that CM-hAMSC-treated R6/2 mice displayed less severe signs of neurological dysfunction than vehicle-treated ones. Indeed, CM-hAMSC treatment significantly reduced and delayed the development of the hind paw clasping response during tail suspension, deficits in rotarod performance, and also delayed the decrease in locomotor activity in an open field test.

The effects of CM-hAMSC treatment on neurological function were reflected in a significant amelioration in brain pathology, including reduction in striatal atrophy, of striatal neuronal intranuclear inclusions, and of the degree of microglial activation that occurs in response to the mutant huntingtin-induced brain damage.

Conclusions Our findings demonstrate the possibility of ameliorating the behavioral and neuropathological abnormalities of symptomatic R6⁄2 mice and underline the potential therapeutic value of CM-hAMSC in Huntington's disease.

The Role of Vitamin K2 in Bone regeneration: from bench to bed side

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Osteoporosis (OP) is a skeletal pathology that determines a reduction in the bone mechanical resistance. It represents the most common bone disease that affects women and men in the elderly. Recently Vitamin K2 (VitK2), a family of natural molecules also known as Menaquinones, received particular attention for its positive effects on bone metabolism and bone mass maintenance. Since an impaired VitK2 nutritional status is correlated with an increased risk of fractures, several studies showed that VitK2 intake significant increases Bone Mineral Density (BMD), bone health and strength in healthy post-menopausal women.

We recently demonstrated that VitK2, in vitro, supports the bone aggregate formation as well as the human Amniotic Stem Cells osteogenic induction, while inhibits human Monocytes (hMCs) osteoclastogenic differentiation, using a 3D dynamic co-culture system which mimics the physiological bone microenvironment.

Based on these in vivo and in vitro studies, an important role of VitK2 in the prevention of fractures and improving bone health in OP patients has been proposed. Most of the studies were conducted on Japanese population where VitK2, at a pharmacological dosage of 45 mg/die, has been revealed to be effective in the prevention and treatment of OP. However, if a lower VitK2 dose could be beneficial for bone health in Caucasian population is unclear.

Thus, the present clinical study aims at evaluating the effectiveness of the low VitK2 dose, approved by the Ministery of Health (180 μd/die), to improve BMD in Italian elderly osteoporotic patients undergoing surgery for femoral neck fracture (FNF).

The study is performed as a randomized, triple-blind, placebo-controlled trial. The hypothesis is that the VitK2 oral intake for 12 months in association with N-Acetyl Cysteine (NAC; antioxidant which should improve VitK2 absorption and bioavailability) is effective in improving BMD and the bone turnover markers.

Therefore, 144 participants (men and women) are randomly assigned to one of four groups (n=36): placebo, 180 μg/die VitK2, 300 mg/die of NAC and VitK2 in association with NAC.

In parallel an in vitro study will aim at better understanding the VitK2 molecular mechanisms using an innovative 3D co-culture system composed of autologous cells.

Bone waste fragments and peripheral blood (PB) samples will be collected from the recruited patients in order to isolate osteoblasts (OBs) and hMCs (osteoclast precursors) and to develop a 3D autologous hOBs-hMCs co-culture system, as an in vitro model to mimic the process of bone matrix deposition and remodelling.

Recently the ability of VitK2 to promote angiogenesis in vitro and to ameliorate vessel of the femoral head in a osteonecrosis-rat model in vivo was demonstrated. Autologous human endothelial progenitors cells (hEPCs) will be also isolated from recruited patients and added to the 3D hOBs-hMCs system in order to better understand the problem of inadequate vascular supply which occurs during bone regeneration.

In conclusion, this study will allow us to demonstrate if VitK2 may influence the speed of bone renewal process in vivo. Moreover, using autologous vascularized 3D hOBs/hOCs/hEPCs constructs, it is potentially useful for optimizing future therapeutic approaches in vitro.

Hypoxia preconditioning of human MSCs: a direct evidence of HIF-1α and Collagen type XV correlation

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Mesenchymal stromal cells (MSCs) hold considerable promise in bone tissue engineering, but their poor survival and potency when in vivo implanted limits their therapeutic potential. For this reason, the study of culture conditions and cellular signals that can influence the potential therapeutic outcomes of MSCs have received considerable attention in recent years. Oxygen (O2) has been demonstrated to be a potent signaling molecule, and O2 tension is one of the crucial factors in the skeletal homeostasis that may be adequately monitored and changed in cell culture systems. Vascular disruption during bone fracture creates a hypoxic environment within the developing callus. Accordingly, the initial stages of repair at the fracture site occur in a hypoxic zone where a heterogeneous cell population including MSCs, osteoblast precursors, mature osteoblasts and osteocytes is present. Therefore, mimicking the natural microenvironment by lowering in vitro O2 tension could provide results closer to what happens in vivo. Moreover, it has been recently demonstrated that short exposure to hypoxic cultural environment could be beneficial for cell expansion before in vivo transplantation for bone tissue regeneration therapy. However, a standard protocol does not exist and the precise mechanism through which hypoxia preconditioning affects the cells remains unclear.
The aim of present study was to explore what happens during the first 48h of hypoxia preconditioning in human MSCs cultured in the X3 Hypoxia Hood and Culture Combo – Xvivo System, by investigating the expression modulation of critical genes which could be good markers of increased osteopotency. In the conditions by us adopted, the cells showed a significant increase of migration without significant signs of apoptosis, cytoskeleton disorganization or ROS accumulation. Interestingly, for the first time we identified Collagen type XV (ColXV), a chondroitin sulphate modified glycoprotein, as a new low O2 tension sensitive gene, and provided a novel mechanistic evidence that directly HIF-1α (hypoxia-inducible factor-1 alpha) mediates ColXV expression in response to hypoxia. By Luciferase reporter assay and Chromatin immunoprecipitation we demonstrated that HIF-1α was specifically recruited at ColXV promoter, in hypoxia-preconditioned hMSCs. This finding, together the evidence that also Runx2, VEGF and FGF-2 expression increased in hypoxia preconditioned hMSCs, is consistent with the possibility that increased ColXV expression in response to hypoxia is mediated by an early network that supports the osteogenic potential of the cells. This adds useful information to understand the role of a still little investigated collagen such as ColXV that we previously proved to be a critical regulatory component of the osteogenic pathway and a prerequisite to promote the subsequent deposition of mineral matrix. Here we 1. identified ColXV as a new marker of successful hypoxia preconditioning, 2. gave further evidence that hypoxia preconditioned hMSCs have greater osteopotency than normal hMSCs, and 3. suggested that the effects of hypoxic regulation of hMSCs activities should be considered before they are clinically applied.

Decellularized Wharton's Jelly from human umbilical cord: a novel scaffold for tissue engineering

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In the last decade, the production and use of decellularized tissues in tissue engineering applications is receiving considerable attention, as the natural extracellular matrix (ECM) can provide necessary physical cues supporting the restoration and development of functional tissues. In relation to bone and osteochondral tissue engineering, we are interested in producing decellularized Wharton's jelly matrix (DWJM) from human umbilical cord to be used as a scaffold to restore the bone/osteochondral microenvironment. WJ is a mucous connective tissue that surrounds the umbilical cord vessels and is covered by a layer of simple amniotic epithelium. This is a very peculiar tissue with several unique biomechanical characteristics related to its biochemical structure.
We have explored the composition of human WJ extracellular matrix and developed a strategy for decellularization based on a previous protocol set up in our lab with reagents preserving ECM structure and composition. Decellularization is a process that allows the DNA removal and should preserve morphology and chemical factors of the processed tissue. In this work, Wharton's jelly decellularization was performed through different methods, including the chemical Latrunculin and the detergent enzymatic strategy (sodium dehoxycholate-SDC- /DNAse) demonstrating that the second method was more efficient in balancing both preservation of tissue structure, composition and cell removal. Masson's trichrome and Alcian blue staining were performed to measure both the efficacy of cells removal and the percentage of glycosaminoglycans (GAGs) content; DNA quantification was carried out to assess the elimination of genetic material.
We are now planning cell culture experiments to investigate the potentiality of DWJM in regenerative medicine applications, and improve the properties of the cells for bone and cartilage tissue repair. For this purpose, the attachment, survival and differentiation ability of human mesenchymal stem cells, osteoblasts and chondrocytes will be tested (a) in X3 Hypoxia Hood and Culture Combo – Xvivo System with 1-3% oxygen tension in order to mimic the physiological conditions, and (b) in the absence of differentiating agents, relying on the potential of the components of the DWJM in supporting the behavior of the cells.

Human muscle precursor cells expanded in hypoxia as promising tool for muscle regeneration

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In children and adults, skeletal muscle engineering could represent a good strategy to treat skeletal muscle defects such as congenital and acquired diseases. One of the big challenges is the choice of the correct cell source for tissue reconstruction, considering the feasibility of isolation and the ability of in vitro expansion. We propose a procedure to extract human muscle precursors cell (hMPC) from fresh and frozen muscle biopsies of different muscle type and we cultured them in different oxygen tension. We have developed a procedure for the recellularization of extracellular matrix derived from quadriceps muscle through injection of human muscle precursors cells using a platelet gel as vehicle. Both for in vitro and ex vivo experiments we performed immunofluorescence and histological analysis. We performed molecular analysis for myogenic genes, assessing the expression in the recellularized samples. Methods Twenty five human muscle biopsies (age from 20 to 65, male and female) from Tibialis and Peroneous muscles were used after informed consent. Biopsies were finely minced and either frozen in liquid nitrogen for later use or straight digested with type I collagenase and trypsin-EDTA. Cells were then cultured at 20% and 5% oxygen. The effect of 1 μM bpV(phen) and 1 μM Q-VD-OPh addition was also assessed. Doubling time analysis, myogenic index evaluation and immunoflorescence staining for Myf5, MyoD, Pax7 and Ki67 were performed. Using a detergent enzymatic method we decellularized quadriceps muscle obtaining an empty scaffold (extracellular matrix) that we engineered with h MPC cells. Results and conclusions. We firstly demonstrated that hMPC from frozen biopsies did not display any difference compared to cells obtained from fresh samples, in terms of proliferation, myogenic marker expression and in vitro myotube formation. Both at 20% and 5% of oxygen tension, unselected cells homogenously expressed CD56 after two or more passages (more than 90% CD56+ cells), without FACS selection. We observed that in hypoxia and after addition of chemicals the doubling time was shorter in respect to normoxia, improving the cell number harvesting, whereas the expression of myogenic markers and the myogenic index did not change according to oxygen condition. We created ex vivo a 3D muscle seeded with hMPC demonstrating their good mobility, proliferation rate and potential regeneration ability. Taking together, these findings may have important implications for clinical application of skeletal muscle tissue engineering.

Inhibin-A and Decorin Secreted by Human Adult Renal Stem/Progenitor Cells Through the TLR2 Engagemen Induce Renal Tubular Cell Regeneration

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Acute kidney injury (AKI) is a public health problem worldwide. Several therapeutic strategies have been made to accelerate recovery and improve renal survival. Recent studies have shown that human adult renal progenitor cells (ARPCs) participate in kidney repair processes, and may be used as a possible treatment to promote regeneration in acute kidney injury. Here, we show that human tubular ARPCs (tARPCs) protect physically injured or chemically damaged renal proximal tubular epithelial cells (RPTECs) by preventing cisplatin-induced apoptosis and enhancing proliferation of survived cells. tARPCs without toll-like receptor 2 (TLR2) expression or TLR2 blocking completely abrogated this regenerative effect. Only tARPCs, and not glomerular ARPCs, were able to induce tubular celi regeneration process and it occurred only after damage detection. Moreover, we have found that ARPCs secreted inhibin-A and decorin following the RPTEC damage and that these secreted factors were directly involved in celi regeneration process. Polysaccharide synthetic vesicles containing these molecules were constructed and co-cultured with cisplatin damaged RPTECs. These synthetic vesicles were not only incorporated into the cells, but they were asso able to induce a substantial increase in cell number and viability. The findings of this study increase the knowledge of renal repair processes and may be the first step in the development of new specific therapeutic strategies for renal repair. A NANOCARE-AKI project will study the regenerative aspect by administering these two chemokines (inhibin-A and decorin) vehicled by synthetic nanovesicles (NVs) in animal models with AKI induced by glycerol infusion in mice and by cisplatin in pigs.

The role of purinergic system in mature and precursor bone cells

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The biological effects of extracellular ATP are mediated by P2Y metabotropic and P2X ionotrophic receptors. Among P2X receptors, the P2X7R subtype exhibits unique molecular, functional and physiological features. P2X7R activation by low ATP concentrations reversibly opens a cation-permeable membrane channel. On the other hand, stimulation with high ATP concentrations induces formation of a large conductance pore permeable to high molecular weight molecules.
The role of P2X7R in bone biology is only in part investigated and it is still not clear whether the net effects on bone are positive or negative. It has been demonstrated that osteoblasts (OBs) and osteoclasts (OCs) express P2X7R, but its participation in the differentiation of mesenchymal stem cells (MSCs) and in the regulation of bone formation and bone resorption is not still completely understood. The aim of this study was to investigate the role of P2X7R in human primary cells including MSCs (from Wharton's jelly, WJ-MSCs, and bone marrow, BM-MSCs) and OBs in terms of ability of the cells to deposit mineral matrix, according to the scheme below:
-Use of culture systems that are close to the physiological bone micro-environment. We cultured the cells in X3 Hypoxia Hood and Culture Combo – Xvivo System to mimic the hypoxic conditions in which the cells grow in vivo (1-3% oxygen pressure);
-Treatment with P2X7R agonist (BzATP), P2X7R antagonists (A740003 or AZ11645373) or apyrase (scavenger of ATP and ADP) to elucidate the role of extracellular ATP in the deposition of mineral matrix;
-Analysis of ectonucleotidases expression and activity to investigate the participation of these enzymes to produce inorganic phosphates for mineralization.

Preliminary data indicate that:
1. After 48h of hypoxia preconditioning, there was no difference in the mRNA expression of P2X7R in WJ-MSCs, but there was a decreased of mRNA expression in BM-MSCs and OBs. No difference in the P2X7R protein (analyzed by Western Blotting) in the three different cell types;
2. The treatment with P2X7R agonist, P2X7R antagonists or apyrase was not toxic to the different cells;
3. Matrix mineralization analyzed by Alizarin Red staining decreased after treatment with P2X7R antagonist only in OB cells. We also demonstrated that the P2X7R protein increased after the exposure of BM-MSCs and OBs, but not WJ-MSCs, to osteogenic medium.
With this planning we aim to elucidate the P2X7R role in the bone context and the potential of P2X7R as a therapeutic target for treatment of bone diseases.

Celector® “the cell chromatohraph” for mesenchymal stem sel evaluation

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A homogeneous, viable and proliferative cell populations is a necessity for stem cell research and for their future clinical application. Stem Sel developed Celector®, the “cell chromatograph”, a novel device for the separation and real-time microscopy of living cells. Celector® tag-less and free-flow separates cells from complex and heterogeneous samples on their native physical properties which are fully maintained and not altered. Celector® applications go from cell isolation to quality-control system as it provides a typical profile depending on the cell components. Samples are injected into a sterile capillary and are eluted through a biocompatible flow: bigger or more dense cells elute first while smaller and lighter cells elute later. A fractions collector is placed at the capillary outlet to collect viable cells for further analysis or use. A real-time optical system allows to obtain live imaging and cells number through a cell count software. In the present study, Celector® technology was used to separate cells from human concentrated bone marrow (BMC). To this end, BMC was diluted in order to achieve a cell concentration of 6-8x106 and we were able to obtain three different fractions named F1, F2 and F3. Morphological analysis, citofluorimetric evaluation of specific markers by FACS, Colony Forming Unit assay (CFU-F) and chondrogenic/osteogenic differentiation were performed in the three fractions. Cellular morphology evaluated after 15 days showed that cells from F1 fraction are similar to BMC. They have a fibroblastoid phenotype and are well organized. Cells from fractions F2 and F3 seems to growth in cluster and looks less organized and more rounded. CFU-F assay performed in all the samples, showed in the majority of the cases evaluated that F1 fraction had a high clonogenic ability to form colonies at day 20, while few colonies were noted in the F2 and F3 fractions. The histological analysis performed by Alcian blue and Alizarin red showed as cells from F1 fraction are able to better differentiate both toward chondrogenesis and osteogenesis compared to the other ones.
In conclusion, in this study Celector® provides an effective separation of stem cells from bone marrow maintaining their native characteristics and avoiding cell manipulation. This allows cell collection for further studies, amplification and potential reuse for stem cell-based applications.

Effect of P2X7 modulation on the survival and aggressiveness of cancer stem cells from human glioblastoma multiforme

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Glioblastoma multiforme (GBM) is the most common and lethal brain tumor in adult humans. The presence of stem-like cells in GBM (GSCs) with high self-renewal, resistance to radio/chemotherapy and invasiveness/migration potential seems to underlie the unfavorable GBM prognosis. An important process in tumor genesis, metastasis, and recurrence, namely epithelial-to-mesenchymal transition (EMT), would favor GBM recurrence. Although still debated in a neuroepithelial context like that of GBM, EMT could induce biochemical changes and a more mesenchymal phenotype in GSCs, enhancing their migration, invasiveness and resistance to apoptosis. We previously demonstrated that ATP and some related derivatives, acting on ionotropic P2X7 receptors (P2X7R) restrain GSC growth, also potentiating the cytotoxic activity of temozolomide (TMZ), a drug currently used in GBM therapy (D'Alimonte et al., Purinergic Signal. 11; 331, 2015). Here, using the same GSCs derived from human surgical GBM specimens, we investigated the effects of P2X7R agonists/antagonists on EMT-associated gene expression and GSC migration/invasion.
GSCs were exposed to Transforming Growth Factor β (TGFβ), a known inducer of EMT process, or 2'(3')-O-(4-benzoylbenzoyl)-ATP (Bz-ATP, 50-200μM), selective P2X7R agonist. TGFβ (5-10 ng/ml) significantly increased mRNAs of selected EMT markers (ZEB1, N-cadherin, Snail) in the 24-48 h following its administration to GSCs, whereas BzATP effect (50-200 μM) was shorter, being significant at 12 and 24 h after cell exposure to the drug, as evaluated by qRT-PCR. Western blot (WB) analysis confirmed this trend, showing an increased expression of EMT markers such as N-cadherin, ZEB, Twist and vimentin by TGFβ and Bz-ATP in the 24-72 h following their administration. Bz-ATP- induced effects were counteracted by the P2X7R antagonist A438079 (10 μM). Interestingly, Bz-ATP, like TGFβ, enhanced GSC migration and invasion assayed by classical scratch and migration (through matrigel-coated transwells) tests slightly affecting cell viability. Finally, we investigated the expression of the two main human P2X7R splicing variants (the full length P2X7RA and the truncated P2X7RB, lacking the carboxylic tail) that may differently affect tumor cell survival. mRNA and protein expression of the two P2X7R isoforms, evaluated by qRT-PCR and Western blot analysis preceded by immune-precipitation, respectively, showed the presence of both isoforms, with a greater expression of P2X7RB, especially in GSCs which we previously showed to be more resistant to the cytotoxic TMZ action. Therefore, we can conclude that in human GSCs: i) activation of P2X7R stimulates the EMT process and cell migration; ii) there may be a prevailing expression of the P2X7RB, that is considered a pro-tumor receptor. Since GBM microenvironment includes elevated levels of ATP required for P2X7R activation, our data support a role of P2X7R in GBM growth and invasiveness.